Effect of M&B 693 and Uleron on the Blood

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Whilst there is little known about the pathology of agranulocytosis, little doubt can be entertained as to the part played by the sulphonamide compounds in its causation. Bigler, Clifton and Werner (1938), Britton and Hawkins (1938), and French (1939), amongst others, have demonstrated that sulphanilamide causes a considerable fall in the number of white blood-cells without the actual appearance of agranulocytosis, whilst, unfortunately, numerous cases of granulocytopenia have been recorded following the use of this drug.

With the advent of M&B 693 it was hoped that there would be less likelihood of similar damage to the blood-cells of the body. Early reports seemed to promise fulfilment of this hope : Evans and Gaisford (1938) found no difference in white-cell counts performed on patients treated for pneumonia with the drug as compared with those treated by routine methods, despite the fact that very heavy dosage of M&B 693 had been employed. Fleming (1938) likewise found that the drug had no deleterious action on the leucocytes, at any rate in vitro. Lloyd, Erskine and Johnston (1938), however, noted a slight but definite depression of the total white blood-cell count with polymorphonuclear leucopenia and a relative lymphocytosis in a few of their patients who were being treated for gonorrhœa. Somewhat similar observations were made by Batchelor et al. (1938), Brown (1939), and Melton and Beck (1939). The latter workers state that M&B 693 did not appear to affect the red blood-cells : Long (1939), however, found that two cases of acute hæmolytic anæmia occurred during administration of the drug at the Hospital of the Rockefeller Institute. Unfortunately, as with sulphanilamide, a number of cases of agranulocytosis following the use of M&B 693 have been recorded.

Following the introduction of M&B 693, heavy dosage of the drug was recommended especially in the early stages of treatment, with the result that many "minor" toxic manifestations (i.e., nausea, vomiting, headache, depression, etc.) were reported. The possibilities of more serious though less obvious toxic manifestations became apparent, and the desirability of evolving a scheme of dosage which would give the optimal clinical results with minimal toxic reactions presented itself. This was of particular importance not only generally, but in view of the fact that many of the patients treated were attending hospital as out-patients. An investigation was accordingly carried out personally with these objects in view. The cases investigated were chiefly out-patients being treated for gonorrhœa. The following are some of the results of this investigation.

The reaction of patients on Uleron therapy was also studied in a smaller series of cases.

PROCEDURE.

Sixty-one male patients were investigated: fifty-nine were suffering from gonorrhœa and two from staphylococcal urinary infection. Of these, fifty were patients treated with M&B 693, seven were treated with Uleron, and four were normal controls. The fifty patients on M&B 693 were divided into three groups A, B, and C :—

Group A.—This consists of forty-four out-patients who had the same dosage over a period of seven days (with occasional slight variations); during the first day they were given 3g. of the drug, whilst 2g. per day were administered thereafter for a further six days.

Group B.—Group B consists of four patients to whom heavier dosage over a more prolonged period was administered as follows :—

(I). Pt.Sh. 42.5g. in thirteen days (in-patient suffering from epididymitis).

(II). Pt.L. 17.5g. in ten days (in-patient suffering from staphylococcal urinary infection and para-urethral abscess).

(III). Pt.B. 24.0g. over a period of twenty-five days, which included seventeen days' interval (out-patient suffering from gonorrhœa)

(IV). Pt.Sc. 45.0g. over a period of twenty-three days, which included three days' interval (out-patient suffering from gonorrhœa).

Group. C.—In two cases, a daily or bi-daily blood investigation was performed over a period of six days following single doses of M&B 693 of 4g. and 2g. respectively.

The patients on Uleron were given 3g. of the drug per day for four days, followed by a period of seven to eight days' rest, when a second "stoss" similar to the first was administered, and, after a further interval of rest as before, a third "stoss" was given. The normal controls were four out-patients who were being treated by routine methods of urethral irrigations and mixed gonococcal vaccine.

Serial total and differential white-cell counts and red-cell counts were made with ordinary standard pipettes, the hæmoglobin being estimated by the Sahli method. In order to lessen the known daily fluctuations in the cell counts as much as possible, the counts were performed as nearly as possible at the same time each day, whilst to ensure the maximum degree of accuracy in the actual white-cell counts themselves, the following points were observed :—

(I). In the estimation of total leucocytes, four preparations were made, and the four counts thus obtained were averaged.

(II). In the estimation of the differential count, at least four hundred unselected white blood-cells were examined, and in many cases considerably more than this number were counted.

Normal readings were taken as follows :—

Hæmoglobin - - - -	99—100 per cent.
Red blood-cells - - - -	5,100,000 to 6,350,000
Total leucocytes - - - -	5,000 to 10,000

Differential Counts :—	Total per cmm.	Percentage of Total Leucocytes.
Neutrophil polymorphonuclear leucocytes -	3,000—6,000	60—70
Lymphocytes - - - -	1,500—2,700	25—30
Monocytes - - - -	350— 800	5—10
Eosinophil polymorphonuclear leucocytes -	150— 400	1— 4
Basophil polymorphonuclear leucocytes -	0— 100	0— 1

Anything above or below these figures was considered to be abnormal.

In the patients taking M&B 693, blood investigations were performed whilst the drug was being taken and for a varying period thereafter up to the end of thirty-two days from the commencement of chemotherapy (and occasionally for an even longer period). In order to ascertain as nearly as possible the exact day on which the maximum effects, if any, were seen, the investigations were carried out on different days on different patients, so that a fairly complete daily series was obtained during the period of examination. As the majority of blood counts were performed up to and including the twenty-second day from the beginning of chemotherapy, the results will be considered in detail only up to that day, but reference will be made to results obtained at an even later period. In the case of Uleron patients, blood investigations were performed before the beginning and at the end of each "stoss" and finally a week after the end of chemotherapy. Investigations were carried out at frequent intervals over a period of two weeks on the control cases.

RESULTS.

Blood Cell Changes with M&B 693.

Group A—Forty-four Patients on the Same Dosage.

Total Leucocytes.

The average total leucocyte count for the forty-four patients showed a distinct drop both during chemotherapy and for some considerable time after discontinuing the drug, reaching its lowest point on the sixteenth day (i.e., nine days after the end of chemotherapy). After this the count rose again. Though the average figure did not reveal a leucopenia at any time, eight individual cases (or just over eighteen per cent. of the series) gave a total leucocyte count of less than 5,000 per cmm. on one or more occasions. Of these, the lowest count coincided with the third day after commencing chemotherapy in one case, the fourth day in two cases, the fifth day in one case, the eighth day in four cases, and the eighteenth day in one case, so that, though the difference was small, the greatest number of cases of leucopenia occurred after the withdrawal of the drug, the maximum number being seen on the day immediately following the end of chemotherapy. The lowest figure recorded was 4,062 on the eighth day on a patient whose original white-cell count had been 9,687.

Differential Leucocyte Counts.

Polymorphonuclear Neutrophil Leucocytes. (Neutrophils).

It became evident that the downward trend in the average total leucocyte count was due to a fall in the number of the neutrophil leucocytes. Here again the lowest total count occurred on the sixteenth day, when a figure of 2,888 cells per cmm. was recorded, so that an actual average neutropenia was observed on that day. Actually approximately thirty-two per cent. of the series showed a neutropenia on one or more occasions, the lowest figure recorded being 1,960 cells per cmm. on the eighth day, on the patient mentioned before as having the lowest total leucocyte count : this was the only occasion on which a count of below 2,000 cells per cmm. was recorded in this series.

Whilst a definite neutropenia was thus observed, the most striking feature in the investigation of the white cells was not the changes in the total neutrophil counts so much as a marked variation in the percentage of neutrophils present.

It was observed time after time that the percentage of neutrophils decreased, and again it was found that the maximum effect appeared on the sixteenth day. Actually approximately eighty-three per cent. of the cases in this group showed a fall in neutrophils on one or more occasions to below sixty per cent. of the total leucocytes : 36.6 per cent. showed a fall to between forty and fifty per cent. of the total leucocytes, whilst 9.7 per cent. of cases actually fell to below forty-five per cent. of the total white cells. The lowest figure recorded was 41.75 per cent. on the eighth day in a patient whose neutrophils had been 64.5 per cent. of the total leucocytes before chemotherapy was commenced.

Lymphocytes.

The average total lymphocyte count showed a fairly steady level throughout, though actually 36.6 per cent. of cases showed a slight lymphocytosis of more than 2,700 cells per cmm. on one or more occasions. 24.4 per cent. were above 3,000 cells per cmm. at some time during the investigation, the highest figure reached being 3,811 in one case on the nineteenth day.

As was to be expected, as a result of the changes in the neutrophils, a marked variation in the percentage of lymphocytes present was seen, increasing as the neutrophils decreased. Thus 76.6 per cent. of the cases showed more than thirty per cent. of lymphocytes in their differential counts on a number of occasions, 24.4 per cent. of the total having more than forty per cent. of lymphocytes at some period. The highest percentage recorded was 45.25 in one case on the sixteenth day.

Though variations in the number of lymphocytes are usually relative, and depend merely on the number of polymorph cells present, absolute increase is not unknown. It is difficult to state where an absolute increase in lymphocytes has occurred in the present series. French (1939), in her investigations on the action of sulphanilamide on the blood, considered that all cases showing a total leucocyte count of 10,000 or more per cmm., and in which the lymphocytes numbered thirty or more per cent., represented a true lymphocytosis. On this basis, 9.7 per cent. of cases in this present series showed an absolute lymphocytosis, though 7.3 per cent. also had a neutrophil count of less than sixty per cent. at the same time.

Mononuclear Leucocytes. (Monocytes).

Though the average number of monocytes present tended to show a slight fall, there was a considerable variation in the findings recorded, and the average number kept well within the limits of normality throughout. Actually in 29.5 per cent. of cases a monocytosis of over 800 cells per cmm. was found, the greatest number being 1,291 on the fourth day in one patient.

Here again, greater variation was seen in the percentage of cells recorded. In forty-five per cent. of the patients in the series, a reading of over ten per cent. of monocytes was observed, the highest being 14.75 per cent. in one case on the fourth day.

Eosinophil and Basophil Polymorphonuclear Leucocytes.
(Eosinophils and Basophils).

Neither the eosinophils nor basophils showed any constant change. The highest recorded figure of the series for the eosinophils was 12.75 per cent. or 1,187 cells per cmm. on the eighth day in one case who, however, tended to show a high eosinophil count throughout.

Hæmoglobin and Red Blood-cells.

Little variation was seen in the hæmoglobin throughout the entire series, though several cases showed a slight fall at some time or another; this was most marked in one patient whose hæmoglobin fell from ninety-one per cent. before chemotherapy was started, to eighty-one per cent. on the sixteenth day. Five days later, however, it had risen to eighty-five per cent. A slight but significant fall was noticed, however, in the average red blood-cell count, and again this was most marked at the fifteenth and sixteenth days, when average counts of less than 5,000,000 red cells per cmm. were recorded. Actually 37.5 per cent. of cases showed a count of less than 5,000,000 red blood-cells at some time or other during the period of investigation. The lowest recorded red-cell count was 4,170,000 on the eighth day on a patient whose count before chemotherapy had been 5,010,000 per cmm. In the majority of cases, the reduction in the number of red blood-cells was of a much more transient nature than that of the white cells.

Group B—Four Patients on Heavier Dosage.

In view of the findings recorded above, it was interesting to note that whilst the patient who had the largest dose of the series (45g. over a period of twenty-three days) showed somewhat similar changes in the white cells to those already observed, these changes were much less pronounced than one would have expected, and the blood picture had returned to normal several days before the end of chemotherapy. There was no subsequent fall in the number of cells during the following eighteen days, and in fact the neutrophils increased to over seventy per cent. of the total leucocytes and remained high during the period of observation mentioned, following the withdrawal of the drug. This patient had not responded to chemotherapy, and it was tempting to believe that possibly the drug was being poorly absorbed (unfortunately, the absorption and excretion were not investigated), and subsequently had little deleterious effect either on the organisms causing the disease, or on the blood-forming organs of the body.

An examination of the effects of the drug on the blood in five patients in Group A who either did not respond to chemotherapy or who showed subsequent relapse, revealed somewhat similar changes in four cases to those noted in the majority of patients; in three of these, however, the changes were of a transient nature only. The fifth patient showed no decrease in the number of the blood cells at all.

An investigation was consequently made in those patients in whom absorption and excretion of the drug had been simultaneously studied with the blood-cell changes, in order to see whether or not the changes in the blood cells varied in proportion to the concentration of the free drug in the blood. There was no constant relationship found between the two (see Table).

The patient who received the second largest dose (42.5g. in fifteen days, Pt. S. in Table) again showed similar changes to those in Group A, the maximum effect on blood cells being noticed eleven days after withdrawal of the drug, when the neutrophils fell to 40.5 per cent. of a total leucocyte count of 5,625, the lymphocyte count rising to 48.25 per cent.

Very similar effects were observed in both of the other cases in this group. It was interesting to note that the effects with the larger doses were not necessarily more pronounced than with the small dosage which had been used for the patients in Group A.

Group C—Two Patients on a Single Dose of M&B 693.

In both of these cases variation was slight, though in one (following 2g.) there was a tendency to irregularity. As this patient's blood-cell counts were just on the border-line of normality at the beginning, it was difficult to be sure that any subsequent change was due to the drug.

Blood Cell Changes with Uleron.

Whilst the number of cases of Uleron-treated patients which was studied was admittedly smaller than those on M&B 693, it was clear that Uleron had an even more marked toxic effect on the white and red blood-cells than M&B 693, and that this effect was more noticeable at the end of the third "stoss" and continued for some days after withdrawal of the drug. The effect on the leucocytes was again chiefly due to a fall in the number of neutrophil polymorphonuclear cells, though the monocytes also showed a greater numerical reduction than with M&B 693. Seventy-one per cent of cases showed leucopenia at some time or other, the lowest figure being 3,437 leucocytes per cmm. at the end of the third "stoss" in one patient where the original leucocyte count had been 8,125 cells per cmm. An absolute neutropenia was seen in forty per cent. of cases, whilst another twenty per cent. showed relative neutropenia, the lowest figures being respectively 1,873 neutrophils per cmm. at the end of the third "stoss" in one case where the original count had been 5,545 neutrophils per cmm., and 41.75 per cent. of the total leucocytes at the end of the first "stoss" in another case, where the original percentage had been 59.75.

No marked changes were seen in the lymphocytes except those consequent upon the neutrophil variations, whilst the basophils and eosinophils varied little: the latter cells were rather low throughout.

The fall in the number of red blood-cells was again evident, but as before, these changes were of a more transient nature than those observed in the leucocytes. Sixty per cent. of cases showed a decrease in the number of the red cells to below 5,000,000 per cmm. at some time or other.

Normal Controls.

No abnormal variations were seen in the blood-cell count or hæmoglobin in the control cases.

DISCUSSION.

An examination of the results obtained in this series of cases leaves little room for doubt that even comparatively small doses of both M&B 693 and Uleron cause considerable damage to the blood-cells of the body. That the red cells do not escape the destructive process is demonstrated by the results obtained in the red-cell counts. This is rather at variance with the observations made by Melton and Beck (1939). Dolgopol and Hobart (1939), however, in a fatal case of agranulocytosis following M&B 693 described by them, found that, whilst the action of the drug on the bone-marrow apparently consisted mainly in the arrest of maturation of the leucopoietic elements, the erythropoietic elements were also considerably affected. It has been mentioned that at least two cases of acute hæmolytic anæmia have been described by Long (1939). The toxic effect, however, would appear to be more marked on the white-cell elements : that there is a definite tendency to a lowering of the total leucocyte count, and that this is due to a fall in the number of neutrophils, has been shown. That a similar phenomenon is observed after the administration of sulphanilamide has been recorded by Britton and Hawkins (1938) and French (1939), so that the action of M&B 693 on the blood cells would appear to be essentially similar to that of sulphanilamide. Whilst the fall in the number of the total leucocytes and the absolute neutropenia in the present series is rather less than that recorded by Britton and Hawkins (1938), the dosage of M&B 693 is considerably smaller than that of sulphanilamide in the investigation made by these two workers. French (1939) found that only 33.3 per cent. of her sulphanilamide-treated cases gave a neutrophil-count below sixty per cent. of the total leucocytes. That M&B 693 has an even more toxic action on the white cells than sulphanilamide would seem to be indicated by the fact that eighty-three per cent. of cases in the present series exhibited a drop at some time or other in the neutrophil-count to below sixty per cent. of the total leucocytes. This relative neutropenia, together with an accompanying relative lymphocytosis, was so marked that one almost came to regard it as a usual feature in patients who were being treated with M&B 693. Lloyd and his associates (1938) noted a slight but definite fall in the total white blood-cell count with polymorphonuclear leucopenia, and a relative lymphocytosis in a few of their patients who were being treated with this drug : unfortunately, they do not state the number of patients in whom the investigation was carried out, nor the percentage showing this phenomenon.

It is difficult to see just why this toxic effect on the red-cell elements on the one hand and the white-cell elements on the other should be more marked in some patients than others, and why in some cases it should proceed to acute hæmolytic

TABLE.—

The relationship between the concentration of free M&B 693 blood, the percentage of acetylated drug present in the blo blood-cell changes.

Patient	Average Conc. of Free M&B 693 in the Blood (Mgms. %)	Average Percentage of Acetylated Drug in the Blood	Blood-Counts Before Chemotherap			
			Total Leucocytes per cmm.	Total Neutrophils per cmm.	Percentage Neutrophils	P per
Group A						
M1238	2.98	18.1	13,750	8,594	62.5	
M1373	4.1	20.0	10,625	7,836	73.75	5,6
M1038	3.66	20.7	10,312	6,085	59.0	5,1
M71	3.02	14.4	9,867	5,982	61.75	5,92
L707	2.5	27.6	10,625	6,481	61.0	5,0
Group B						
Pt. L	2.2	18.3	16,250	12,317	75.8	6,1
Pt. S	3.67	28.2	7,500	5,362	71.5	5,090,

NOTE.—
Absorption and Excretion of M&B 693 was studied by Werner's (1939) method.

BLOOD-COUNTS AFTER CHEMOTHERAPY				RESULTS	REMARKS
Lowest Total Leucocytes per cmm.	Lowest Total Neutrophils per cmm.	Percentage Neutrophils	Lowest R.B.C. per cmm.		
7,187	3,054	42.5	5,200,000	Failure	Showed a decrease in number once only (4th day).
9,187	4,061	56.5	5,420,000	?Relapse	Showed a decrease twice only (4th and 8th days).
6,562	3,248	49.5	—	Cure	Decrease on the 3rd day from beginning of chemotherapy.
4,062	1,960	48.25	6,600,000	Cure	Decrease once as here, slight on 20th day again.
6,875	3,139	45.66	5,000,000	Cure	Decrease five times —most marked on 14th day.
11,625	4,312	46.0	5,120,000	Cure	Decrease several times, including once beyond the 23rd day from commencement of "693."
4,625	2,278	40.5	5,380,000	Cure	Decrease on 11th day after finishing chemotherapy

anæmia or agranulocytosis. That it unfortunately does, however, has already been mentioned. This disturbance does not seem to depend upon the concentration of the drug in the blood, as patients with low blood concentrations may show an even more marked blood-cell disturbance than those cases where the concentration is comparatively high (see Table). In the fatal case recorded by Dolgopol and Hobart (1939), the highest blood concentration was ten milligrammes per hundred c.c., but other patients have shown concentrations of a much higher standard even than this without apparent ill-effect. Most of the patients in whom agranulocytosis has been observed, have had comparatively high dosage of the drug, and it would seem that this plays an important part in the pathological process. A comparison of an analysis of a number of reports of recorded cases of granulopenia following chemotherapy with M&B 693 with those following sulphanilamide reveals the fact that, whilst the average dose of M&B 693 administered in these cases is similar to that of sulphanilamide, the average period of administration is rather less than for the latter drug : it is suggestive that the total dosage of M&B 693 is of even more importance than the period of administration. This finds support in two cases of granulopenia recorded by Briggs (1939) : M&B 693 was given over an equal period of nine days to each patient; one case, however, had 60g. of the drug, whilst the other had 49g. : the patient with the larger dose manifested the more severe symptoms. It is possible that an undue sensitivity to the drug is exhibited in these cases, whilst the toxic action of the causative organism on the blood-forming tissues undoubtedly plays a part in some of the more serious conditions treated (Colebrook, 1939).

It appears to be impossible to foretell just which patient is liable to develop a serious blood dyscrasia and which is not. That the onset of agranulocytosis has been of such dramatic suddenness that even repeated blood-cell counts have failed to indicate its approach has, unfortunately, been recorded with sulphanilamide (Young, 1937). Nevertheless, a repeated blood-cell investigation should form an integral part of sulphonamide chemotherapy, especially where a dosage of 20g. or over (Pringle et al., 1940) is being given to a patient suffering from a condition likely itself to damage the bone-marrow. A number of other factors are significant in recorded cases of agranulocytosis, and in particular it would seem to be of first importance to keep a close watch on the blood-cells of those patients who show undue signs of toxicity or an atypical response to the drug. That patients who do not show a rapid response to chemotherapy are unlikely to respond at all has been the experience of most workers. In such patients, it seems evident that chemotherapy should be stopped and other means of treatment instituted.

With regard to the cell-counts themselves, it has been demonstrated that the detrimental effect of M&B 693 continues for some days after withdrawal of the drug, and that the maximum toxic effect is often seen during this period : in the investigation described, the greatest effect was witnessed about nine days after cessation of chemotherapy and sometimes even later. This delayed effect has been the experience of other workers also (Barnett et al., 1939), (Graham et al., 1939), (Agranat et al., 1939, and Briggs, 1939). It is important, therefore, that where

large dosage of the drug has been employed, and especially if there have been any signs of undue toxicity or unusual response to chemotherapy, the blood investigations should be continued for at least ten to fourteen days after the drug has been stopped.

SUMMARY.

(1) M&B 693 and Uleron, in common with other sulphonamides, cause a fall in the number of the red and white blood-cells of the body. The effect is greatest on the neutrophils and is more marked with Uleron.

(2) This effect continues after withdrawal of the drugs and may reach its maximum during this period.

(3) The effect on the blood-cells is not proportional to the concentration of the free drug in the blood, and differences must often be attributed to individual variations in the susceptibility of the blood-forming tissues.

(4) Repeated blood-cell counts should be performed on patients taking these drugs, both during chemotherapy and for ten to fourteen days after withdrawal of the drug, especially where large dosage has been employed or where toxic symptoms or atypical response to chemotherapy have been observed.

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